

Examiner states that the present application fails to comply with the requirements of 37 CFR 1.821 through 1.825 as the sequence listing is incomplete, and reference is made to the sequence on page 25, line 19. Applicant notes that the sequence on page 25, line 19 is SEQ ID NO:3. The paragraph on page 25 has been amended accordingly. In SEQ ID NO:3 the designation of "C/T" has been amended to read "y" using accepted nucleic acid symbols. Therefore, Applicant submits that the application is in compliance with 37 CFR 1.821-1.825.

### **Objection under 35 U.S.C. §112**

Claims 32-37 have been objected to under 35 USC 112 as lacking written support from the specification. Applicant respectfully traverses this objection.

The subject matter of claims 32 and 33 is supported within the present specification, for example on page 33-34 (Example 3), and Figures 5-7. This example relates to identifying an 87 base pair deletion identified in *epep* plants. As the 87 nucleotide fragment is present in *EpEp* plants, this sequence may be used to identify and distinguish the genotypes that comprise or lack the fragment. The 87 base pair sequence is defined in Figure 5 (OX347(Ep)) and comprises nucleotides 1524-1610 of SEQ ID NO:2. One of skill in the art would readily understand that any fragment within the 87 base pair region may be used to distinguish between *EPEP* and *epep* genotypes, and examples of such probes and assays are provided. For example primers of 20 nucleotides in length (SEQ ID NO:4-9), as disclosed on page 33, line 13 to page 34, line 12. These primers were used to distinguish the two genotypes as shown in Figures 6 and 7.

Applicant has amended page 34, line 2, to remove reference to SEQ ID NO:20, as there is no SEQ ID NO:20, and to indicate that the 87 base pair region identified in Figure 5, that exists in OX347(Ep) and that is deleted in OX312(ep), corresponds to nucleotides 1524-1610 of SEQ ID NO:2.

Applicant therefore submits that the subject matter of claim 32-37 has support within the specification as originally filed and does not constitute new matter. Removal of the objection under 35 USC 112 against claims 32-37 is requested.

Claims 3, 4, 17 and 25 have been objected to under 35 USC 112.

Claims 3 and 4 have been amended so that the objected to phrase now reads "consisting of". Applicant notes that claims 17 and 25 depend from claim 3, and respectfully requests that the objection against claims 3, 4, 17 and 25 under 35 USC 112 be removed.

### **Objection under 35 U.S.C. §102**

Claims 3 and 4 have been objected to under 35 USC 102(a) in view of Huangpu et al, and 35 USC 102(b) in view of Sigma Chemical Company 1990 catalogue.

Applicant submits that as a result of the amendment to claims 3 and 4, that the subject matter of these claims clearly differentiates both Huangpu et al., and the Sigma Chemical Company 1990 catalogue.

It is requested that the objection to claims 3 and 4 under 35 USC 102(a) and (b) be removed.

ALLOWABLE SUBJECT MATTER

Examiner has indicated that claims 1, 2, 7-16, 18-24 and 26-31 are allowed

It is respectfully submitted that the above-identified application is now in a condition for allowance and favourable reconsideration and prompt allowance of these claims are respectfully requested. Should the Examiner believe that anything further is desirable in order to place the application in better condition for allowance, the Examiner is invited to contact the applicant's undersigned attorney at the telephone number listed below.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

Formal drawings are submitted herewith.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted

By \_\_\_\_\_

Mary J. Wilson  
Reg. No. 32,955

MJW:tat

1100 North Glebe Road  
8<sup>th</sup> Floor  
Arlington, Virginia 22201-4714  
Telephone: (703) 816-4000  
Facsimile: (703) 816-4100

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

The paragraph from page 25 to 26 has been amended as follows:

High seed coat peroxidase (*EpEp*) soybean cultivar Harosoy 63 plants were grown in field plots outdoors. Pods were harvested 35 days after flowering and seeds in the mid-to-late developmental stage were excised. The average fresh mass was 250 mg per seed. Seed coats were dissected and immediately frozen in liquid nitrogen. The frozen tissue was lyophilized and total RNA extracted in 100 mM Tris-HCl pH 9.0, 20 mM EDTA, 4% (w/v) sarkosyl, 200 mM NaCl, and 16 mM DTT, and precipitated with LiCl using the standard phenol/chloroform method described by Wang and Vodkin (1994). The poly(A)<sup>+</sup> RNA was purified on oligo(dT) cellulose columns prior to cDNA synthesis, size selection, ligation into the λ ZAP Express vector, and packaging according to instructions (Stratagene). A degenerate oligonucleotide with the 5' to 3' sequence of TT(C/T)CA(C/T)GA(C/T)TG(C/T)TT(C/T)GT (SEQ ID NO:3) was 5' end labelled to high specific activity and used as a probe to isolate peroxidase cDNA clones (Sambrook *et al.*, 1989). Duplicate plaque lifts were made to nylon filters (Amersham), UV fixed, and prehybridized at 36°C for 3 h in 6 x SSC, 20 mM Na<sub>2</sub>HPO<sub>4</sub> (pH6.8), 5 x Denhardt's, 0.4 % SDS, and 500 µg/mL salmon sperm DNA. Hybridization was in the same buffer, without Denhardt's, at 36°C for 16 h. Filters

were washed quickly with several changes of 6 x SSC and 0.1 % SDS, first at room temperature and finally at 40°C, prior to autoradiography for 16 h at -70°C with an intensifying screen.

The paragraph from page 33 to 34 has been amended as follows:

Primers were designed from the DNA sequence to compare *EpEp* and *epep* genotypes by PCR analysis. Figure 6 shows PCR amplification products from four different primer combinations using OX312 (*epep*) and OX347 (*EpEp*) genomic DNA as template. The primer annealing site for prx29+ begins 182 bp upstream from the ATG start codon; the remaining primer sites are shown in Figure 1. Amplification with primers prx2+ and prx6-, and with prx12+ and prx10- produced the expected products of 1.9 kb and 860 bp, respectively, regardless of the *Ep/ep* genotype of the template DNA. However, PCR amplification with primers prx9+ and prx10-, and with prx29+ and prx10- generated the expected products only when template DNA was from plants carrying the dominant *Ep* allele. When template DNA was from an *epep* genotype, no product was detected using primers prx9+ and prx10- and a smaller product was amplified with primers prx29+ and prx10-. The products resulting from amplification of OX312 or OX347 template DNA with primers prx29+ and prx10- were directly sequenced and compared. The polymorphism is due to an 87 bp deletion occurring within this DNA fragment in OX312 plants, as shown in Figure 5 ([SEQ ID NO:20] corresponding to nucleotides 1524 to 1610 of SEQ ID NO:2). This deletion begins nine bp upstream from the translation start codon and includes

78 bp of sequence at the 5' end of the open reading frame, including the prx9+ primer annealing site.

**In the claims:**

Claims 3 and 4 have been amended as follows:

3. (twice amended) An isolated DNA molecule comprising the nucleotide sequence [defined by] consisting of nucleotides 1533-4700 of SEQ ID NO:2.
  
4. (twice amended) The isolated DNA molecule of claim 3 comprising the nucleotide sequence [defined by] consisting of nucleotides 1-4700 of SEQ ID NO:2.